BANANA GUARD

Protocols

Using kits

Miniprepping

(Adjusted protocol based on Thermo Scientific GeneJET Plasmid Miniprep Kit instructions)

- Add to the pelleted cells 250 µL of Resuspension Solution, invert tube.
- Add 250 µL of Lysis Solution and invert tube.
- Add 350 µL of Neutralization Solution and invert tube.
- Centrifuge 5 minutes.
- Transfer the supernatant to the Thermo Scientific GeneJET Spin Column.
- Centrifuge 1 minute.
- Add 500 µL of Wash Solution and centrifuge for 30-60 s.
- Discard the flow-through and centrifuge empty column for 1 minute.
- Repeat washing step once
- Transfer the column with the DNA attached to the membrane into a new labelled tube.
- Add 25 µL of nuclease-free water to the column and incubate 5 minutes.
- Centrifuge 2 minutes.

*All steps should be carried out at room temperature. All centrifugations should be carried out in a centrifuge at ≥ 12 000 x g

PCR purification kit

(Adjusted protocol based on Thermo Scientific GenJET PCR Purification Kit protocol)

All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g

- Add a 1:1 volume of Binding Buffer to completed PCR mixture. Mix thoroughly.
- Transfer up to 800 µL of the solution to the GeneJET purification column.
- Centrifuge for 30-60 s. Discard the flow-through.
- Add 700 µL of Wash Buffer to the GeneJET purification column. Centrifuge for 30-60 s.
- Discard the flow-through and place the purification column back into the collection tube.
- Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove any residual wash buffer.
- Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube.
- Add 25 µL of pre heated water (nuclease-free) to the center of the GeneJET purification column membrane and centrifuge for 1 min.

Agarose gel purification kit

(Adjusted protocol from PCR Clean-up protocol from Macherey-Nagel)

- Take a clean scalpel to excise the DNA fragment from an agarose gel.
- Determine the weight of the gel slice and transfer it to a clean tube.
- For each 100 mg of agarose gel < 2 % add 200 µL Buffer NTI.
- Incubate sample for 5–10 min at 50 °C. Vortex the sample briefly every 2–3 min until the gel slice is completely dissolved!
- Place a NucleoSpin® Gel and PCR Clean-up Column into a Collection Tube (2 mL) and load up to 700 µL sample.
- Centrifuge for 30 s at 11,000 x g. Discard flow-through and place the column back into the collection tube.
- Add 700 µL Buffer NT3 to the NucleoSpin® Gel and PCR Clean-up Column.
- Centrifuge for 30 s at 11,000 x g. Discard flow-through and place the column back into the collection tube.
- Repeat previous washing step
- Centrifuge for 1 min at 11,000 x g to remove Buffer NT3 completely
- Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube.
- Add 25 µL pre-heated water (nuclease-free) and incubate at room temperature for 10 min.
- Centrifuge for 1 min at 11,000 x g.